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Synthesis of [^{18}F]RGD-K5 by catalyzed [3 + 2] cycloaddition for imaging integrin $\alpha_v\beta_3$ expression in vivo

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ABSTRACT

In the last few years click chemistry reactions, and in particular copper-catalyzed cycloadditions have been used extensively for the preparation of new bioconjugated molecules such as ^{18}F-radiolabeled radiopharmaceuticals for positron emission tomography (PET). This study is focused on the synthesis of the Siemens imaging biomarker [^{18}F]RGD-K5. This cyclic peptide contains an amino acid sequence which is a well known binding motif for integrin $\alpha_v\beta_3$ involved in cellular adhesion to the extracellular matrix. We developed an improved "click" chemistry method using Cu(I)-Monophos as catalyst to conjugate [^{18}F]fluoropentyne to the RGD-azide precursor yielding [^{18}F]RGD-K5. A comparison is made with the registered Siemens method with respect to synthesis, purification and quality control. [^{18}F]RGD-K5 was obtained after 75 min overall synthesis time with an overall radiochemical yield of 35% (EOB). The radiochemical purity was >98% and the specific radioactivity was 100–200 GBq/μmol at the EOS.

1. Introduction

Integrins are heterodimeric ($\alpha - \beta$) transmembrane proteins expressed at the cell surface that are involved in cellular adhesion to the extracellular matrix [1,2]. They stimulate vascular endothelial cell migration and invasion, regulate their growth, survival and differentiation and they serve as receptors for a variety of extracellular matrix proteins including vitronectin, fibronectin, fibrinogen and osteopontin. They are involved in many biological processes such as angiogenesis, thrombosis, inflammation, osteoporosis and cancer, playing a key role in many severe human diseases [3–5]. So far, 18 α and 8 β subunits of integrins have been identified: they form 24 heterodimers, each with distinct ligand binding properties. Among the integrin superfamily, $\alpha_v\beta_3$ and $\alpha_5\beta_1$ integrins, targeted by the RGD sequence, play a pivotal role in the formation of new blood vessels in tissues (angiogenesis) [6–9]. $\alpha_v\beta_3$ and $\alpha_5\beta_1$ integrins are overexpressed on activated endothelial cells during physiological and pathological angiogenesis [10].

Since $\alpha_v\beta_3$ integrin is expressed on tumor cells of various types (melanoma, glioblastoma, ovarian and breast cancer) where it is involved in the processes that govern metastasis, it represents an attractive target for cancer therapy and has stimulated ongoing research to define high affinity ligands [11,12].

RGD containing integrin ligands potentially have a large number of medical applications ranging from noninvasive visualization of integrin expression in vivo to the synthesis of functionalized biomaterials. Over the past decade, a variety of radiolabeled cyclic peptide antagonists with structures based on the RGD sequence have been evaluated as integrin $\alpha_v\beta_3$-targeted radiotracers [13–15]. The PET tracers [^{18}F]Galacto-RGD, [^{18}F]AH111585 and [^{18}F]RGD-K5 are currently under clinical investigation for visualization of integrin $\alpha_v\beta_3$ expression in cancer patients [16–21].

Due to its favourable β-energy and half-life, fluorine-18 is the most frequently used radionuclide in PET. However, rapid and direct non carrier-added ^{18}F-labeling of complex biomolecules such as peptides is not straight forward. The main approach to label peptides with ^{18}F is via fluorination of prosthetic groups which are then conjugated to the biomolecule [22–24]. [^{18}F]Galacto-RGD, a glycosylated cyclic pentapeptide, is labeled via ^{18}F-acylation with 4-nitrophenyl-2-[^{18}F] fluoropropionate [25]. The acylation methodology is however complex and time consuming. Synthesis of [^{18}F]Galacto-RGD via this prosthetic group method, requires a total synthesis time of about 200 min of which the production of the ^{18}F-prosthetic group takes about 130 min [25]. Another strategy, which has been applied for the synthesis of [^{18}F]AH111585, involves chemoselective oxime

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formation between the aminooxy functionality of the peptide and the carbonyl group of the ^{18}F-labeled aldehyde prosthetic group 4-[^{18}F] fluorobenzaldehyde [26,27]. Introduction of fluorine-18 can also be achieved using the [^{18}F]AlF method in which the ([Al^{18}F]$^{2+}$)-complex is chelated to macrocyclic compounds like 1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA) which can be stably attached to a peptide [28]. For thermostable peptides, the labeling can be performed in one step. Two steps are necessary for heat-labile proteins and peptides where first the binding ligand is labeled with Al^{18}F at elevated temperatures, which is then coupled to the protein [29]. Another inorganic approach for labeling of biomolecules with fluorine-18 is the silicon-fluoride acceptor (SiFA) labeling methodology which uses silicon-containing prosthetic groups for the fluorination of peptides. To overcome the major problem of the in vivo instability of the Si-^{18}F bond, bulky alkyl groups need to be introduced in the silicon-binding building block resulting in excessive lipophilicity which in turn needs to be reduced by introduction of lipophilicity-reducing auxiliaries [30].

Recently, the copper(I)-catalyzed Huisgen 1,3-dipolar cycloaddition reaction (CuAAC) between terminal alkynes and azides resulting in 1,4-disubstituted 1,2,3-triazoles [31,32] has found its way in medicinal [33] and radiopharmaceutical chemistry [34]. The main advantages of this 'click chemistry' approach are selectivity, reliability and short reaction times under mild reaction conditions [35,36]. The ^{18}F-labeling of peptides has been the area that has benefited the most from click chemistry [37,38]. The additional advantage of this chemistry is that there is no need of protective groups when labeling peptides. Both alkynes [37] and azides [38] have been radiolabeled with fluorine-18 to produce ^{18}F-peptides.

As a result of a collaboration between the PET-centers in Leuven (Belgium), Groningen (The Netherlands) and Siemens (MIBR, Los Angeles, USA), we report an improved and simplified procedure to prepare [^{18}F]RGD-K5 using a Cu-catalyst based on the phosphoramidite ligand Monophos [39]. This paper describes in detail the optimized radiosynthesis and QC procedure of [^{18}F]RGD-K5, and compares it with the registered method by Siemens [18–40]. The efficient radiosynthesis procedure can generally be applied for other click reactions using [^{18}F]fluoroalkynes as prosthetic group.

2. Materials and methods

2.1. General

Reagents and solvents were obtained from commercial suppliers (Aldrich, Fluka, Sigma, and Merck) and used without further purification. The RGD-K5 azide precursor and the authentic reference material ^{19}F-RGD-K5 (='cold' standard) were prepared by Siemens. For radiolabeled compounds, radioactivity detection on TLC was performed with Cyclone phosphor storage screens (multisensitive, PerkinElmer). These screens were exposed to the TLC strips and subsequently read out using a Cyclone phosphor storage imager (PerkinElmer, Netherlands) and analyzed with OptiQuant software. HPLC analysis was performed on a LaChrom Elite Hitachi HPLC system (Darmstadt, Germany) connected to a UV spectrometer (Waters 2487 Dual γ absorbance detector). For the analysis of radiolabeled compounds, the HPLC eluate after passage through the UV detector was led over a 3 in. NaI(Tl) scintillation detector (Wallac, Turku, Finland) connected to a multi channel analyzer (Gabi box, Raytest, Straubenhardt, Germany). The output signal was recorded and analyzed using a GINA Star data acquisition system (Raytest, Straubenhardt, Germany).

2.2. Production of 5-[^{18}F]fluoro-1-pentyne and [^{18}F]RGD-K5

A solution of pent-4-ynyl-4-methylbenzenesulfonate (20–25 mg, 84–105 μmol) in 0.8-1 mL anhydrous 1,2-dichlorobenzene was added to the Kryptofix 2.2.2 (20 mg)/K^{18}F residue containing 5 mg of K_2CO_3 and the mixture was heated for 10 min at 110 °C to provide [^{18}F] fluoropentyne which was simultaneously distilled with a gentle flow of helium to a second reactor containing the click reaction mixture. The click reaction mixture contained 0.1 mg RGD-K5 azide precursor(2-((2S,5R,8S,11S)-8-(4-((3S,4S,5R,6R)-6-((2-azidoacetamido) methyl)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxamido) butyl)-5-benzyl-11-(3-guanidinopropyl)-3,6,9,12,15-pentaoxo-1,4,7,10,13-pentaazacyclopentadecan-2-yl)acetic acid; TFA salt) in the presence of 0.2 mg phosphoramidite Monophos (N,N-dimethyldinaphto[2,1-d: 1,2-f][1,3,2]dioxaphosphepin-4-amine) in 0.1 mL DMSO, 1 mol% (0.05 mg) $CuSO_4.5H_2O$ (reduced to Cu(I) with 5 mol% (0.25 mg) sodium ascorbate), in 0.25 mL EtOH and 0.25 mL CH_3CN (Table 1). The subsequent conversion to radiolabeled [^{18}F] RGD-K5 was followed by radio-TLC (R_f [^{18}F]RGD-K5 = 0.4 (eluent: MeOH/H_2O 2:1)). After reacting at room temperature for 10 min, the crude [^{18}F]RGD-K5 was diluted with 1.5 mL of 0.025 M Na_2HPO_4 pH 7 and purified by semi-preparative RP-HPLC using an XBridge C_{18} column (5 μm, 4.6 mm × 150 mm column, Waters) eluted with 0.025M Na_2HPO_4 pH 7.0 and EtOH 88/12 at a flow rate of 1 ml/min. UV detection of the HPLC eluate was performed at 254 nm. [^{18}F]RGD-K5 was collected after 20–25 min (Fig. 2). On average, about 11 GBq (n = 24, ranging from 3.7 to 19.5 GBq) of purified [^{18}F]RGD-K5 was collected in a 1.3–2.6 mL volume (mobile phase). This HPLC-purified fraction was diluted with preparative HPLC mobile phase and passed through an apyrogenic 0.22 μm membrane filter (Millex®-GV, Millipore, Ireland). A final solution of 370 MBq/mL was obtained by further dilution with saline which was passed through the same membrane filter.

2.3. Quality control procedures

Quality control procedures for [^{18}F]RGD-K5 are based upon the current requirements for radiopharmaceuticals laid out in the European Pharmacopoeia [41]. A more detailed description on the materials and methods of these QC tests can been previously described [42].

The radiochemical identity of [^{18}F]RGD-K5 is checked using an analytical HPLC system consisting of an XBridge C_{18} column (3.5 μm, 3 mm × 100 mm; Waters) eluted with 0.025 M Na_2HPO_4 (pH 7) and CH_3CN (90:10 v/v) at a flow rate of 0.8 mL/min. UV detection of the HPLC eluate is performed at 210 nm (Fig. 3). The radiochemical identity of [^{18}F]RGD-K5 is confirmed using the ^{19}F-RGD-K5 standard as an external reference material. After injection and analysis of a solution of the reference material RGD-K5, a blank injection of preparative HPLC mobile phase is performed. The retention time of [^{18}F]RGD-K5 should be the same (±10%) as the retention time observed for the RGD-K5 reference standard. The radiochemical purity and specific activity are analyzed using the same HPLC system. The total of radiolabeled side products should be ≤5%. Rather than setting a lower limit for specific activity, the maximum mass of RGD-K5 which is administered to a patient is limited to <96 μg and the mass of the RGD-K5 azide precursor should be <5 μg per administered dose, these limits were defined in relation to toxicity tests findings performed by Siemens. Residual solvent analysis is performed using GC (direct injection). For the residual class 2 solvent acetonitrile a limit of 4.1 mg per patient dose is set as described in the European Pharmacopoeia. 1,2-Dichlorobenzene is not described in the European Pharmacopoeia but has a no observed adverse effect level (NAOEL) of 120 mg/kg/day in rats which is considerably higher than for chlorobenzene (27 mg/kg/day). For chlorobenzene the European Pharmacopoeia sets a limit of 3.1 mg per day. In order to have a safety margin we have therefore set a limit of 1 mg of o-DCB per injected dose. To be safely administered to the patient, the amount of residual ethanol should be <10% v/v. The drug product pH should be in the range 5–8. Testing of the integrity of the filter that is used for sterile filtration is done by bubble point determination. The bubble

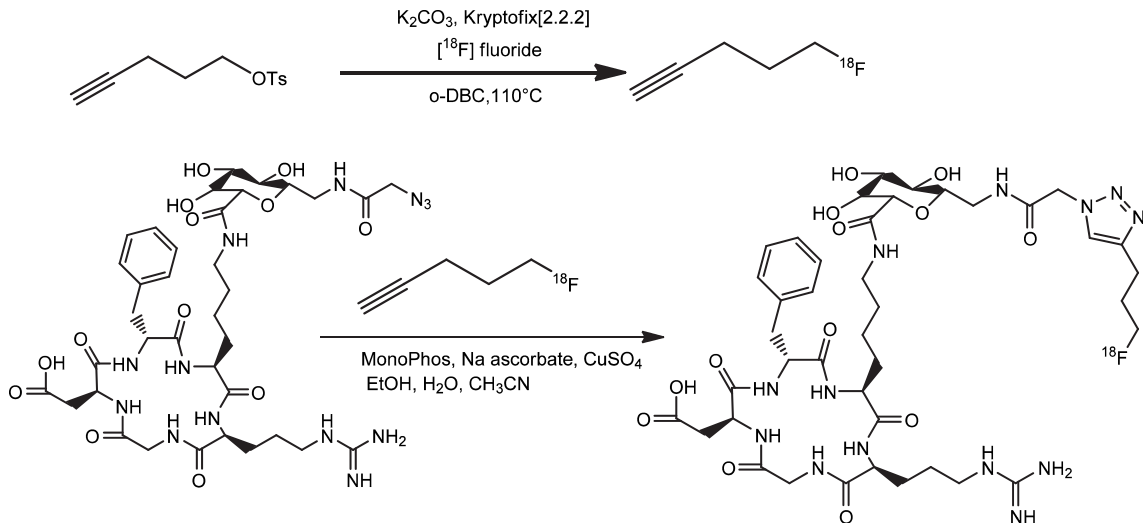
Table 1
Main differences in materials and methods between the optimized [^{18}F]RGD-K5 production procedure as described in this article and the original Siemens protocol.

	Optimized protocol	Siemens protocol
Radiosynthesis of [^{18}F]fluoropentyne		
Pentynyl tosylate	20–25 mg	20–25 mg
Solvent	1 mL o-DCB	0.8 mL CH_3CN
Reaction temperature	110 °C	110 °C
Trapping of [^{18}F]pentyne	Room temperature	−10 °C
Click reaction: radiosynthesis of [^{18}F]RGD-K5		
RGD-K5 azide	0.1 mg	4 mg
Na-ascorbate	0.25 mg	40 mg
$CuSO_4$	0.05 mg	15 mg
Cu(I) Ligand	0.2 mg Monophos in 0.1 mL DMSO	15 mg TBTA
Solvent	EtOH, CH_3CN	EtOH, water, CH_3CN
Reaction temperature	Room temperature	Room temperature
HPLC purification		
Method	Isocratic	Stepwise gradient
Column	XBridge C_{18} (5 μm,4.6 × 150 mm)	Macherey-Nagel Nucleosil C18 (5 μm, 10 × 250 mm)
Mobile phase	0.025 M Na_2HPO_4 pH 7/EtOH	Water/0.05% TFA 5′
	88/12 v/v	5% CH_3CN in water/0.05% TFA 5′
		10% CH_3CN in water/0.05% TFA 5′
		18% CH_3CN in water/0.05% TFA
Flow rate	1 mL/min	5 mL/min
Formulation	Dilution to <10% EtOH	C_{18} Sep Pak to remove CH_3CN
QC		
Method	Isocratic	Stepwise gradient
Column	XBridge C_{18} (3.5 μm, 3 × 100 mm)	Gemini C18 (5 μm, 4.6 × 150 mm)
Mobile phase	0.025 M Na_2HPO_4 pH 7/CH_3CN	Water/0.1% TFA
Flow rate	90/10 v/v	CH_3CN/0.1% TFA
	0.8 mL/min	2 ml/min
Radiochemical yield (EOB)	35%	20%
Total synthesis time	70 min	90 min

point pressure for the particular filter used should be ≥3.45 bar. Determination of the radionuclide identity and endotoxin and sterility testing are performed post batch release. Since $CuSO_4$ is being used in the manufacturing process of the radioligand, the finished drug product should be tested for residual levels of the metal reagent. This is done using inductively coupled plasma mass spectrometry (ICP-MS).

3. Result and discussion

Using our optimized click reaction condition we improved and simplified the original registered Siemens production method. Scheme 1 shows our optimized two-steps radiosynthetic route to yield [^{18}F]RGD-K5. In a first step the labeling synthon 5-[^{18}F]fluoro-1-pentyne was prepared via nucleophilic fluorination of pentynyltosylate with anhydrous [^{18}F]KF-cryptate at 110 °C in o-DCB. The ^{18}F-labeled pentyne (Bp = 76 °C) was isolated from the tosyl precursor and unreacted $^{18}F^-$ via distillation and was, without further purification, trapped in a receiving vial containing the RGD-K5 azide precursor. Effluent gasses that escaped from the receiving vial were collected in a balloon. We choose a high boiling point solvent (o-DCB, 179 °C) instead of acetonitrile (82 °C), the solvent that was used by Siemens, to prevent co-distillation of the solvent. Acetonitrile co-distilling to the click reaction mixture was found to decrease the yield of the click reaction and the efficiency of the HPLC purification. The distilled [^{18}F]fluoropentyne was efficiently trapped at room temperature in the click reactor (90%). Radiometric detection showed that the amount of [^{18}F]fluoropentyne in the receiving vial saturated after about 10 min.



Scheme 1. A) Production of 5-[^{18}F]fluoro-1-pentyne: nucleophilic substitution between pentynyl tosylate and anhydrous [^{18}F]fluoride in ortho-dichlorobenzene (o-DCB). B) MonoPhos Cu(I)-catalyzed Huisgen cycloaddition of [^{18}F]fluoropentyne with the RGD-K5 azide precursor resulting in the 1,4-disubstituted triazole [^{18}F]RGD-K5.

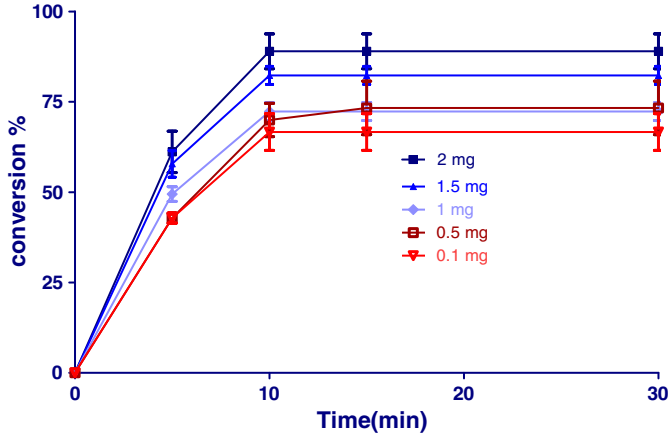


Fig. 1. Radiochemical conversion of the click reaction as a function of time and amount of RGD-K5 azide precursor.

The phosphoramidite ligand Monophos showed to be an excellent ligand for complexation of Cu(I) in order to catalyze the click reaction [39] and is far superior to the previously reported ligand TBTA which is used in the Siemens method [18,43]. Previously we have shown in model systems that the Monophos approach dramatically increased the radiochemical yield. Using 0.05–0.1 mg amounts of benzyl azide conversion yields were 54–99% allowing 10 min for the click reaction. An amount of 0.2 mg Monophos proved to be sufficient to achieve these conversions [44]. In absence of the Monophos ligand only minor conversion to the triazole product [^{18}F]RGD-K5 was observed. Further reaction optimization was performed by varying the amount of RGD-K5 azide precursor from 0.1 mg to 2 mg (Fig. 1). By replacing the TBTA with 0.2 mg Monophos, the amount of azide precursor could be reduced from 4 mg to 0.1 mg and the amount of $CuSO_4$ from 15 mg to 0.05 mg. So far 1 mol % of $CuSO_4$ in the presence of 1.1 mol % Monophos showed sufficient catalytic effect within 15 min to produce [^{18}F]RGD-K5 in an excellent radiochemical yield of 35% starting from only 0.1 mg azide precursor. Usually, as in the Siemens method, large amounts of >1 mg peptide are used without any additional catalysts beside Cu(I). As a result of the dramatic acceleration of the click reaction by using Monophos, we were able to produce [^{18}F]RGD-K5 in good yield with substantially less amount of the reactants sodium ascorbate, $CuSO_4$ and azide precursor (Table 1).

The crude click reaction mixture was purified using semi-preparative HPLC. Before injection, the mixture was diluted with phosphate buffer pH 7 to adjust the pH to that of the mobile phase resulting in sharper peaks. We evaluated different sizes and types of columns and found that the Waters XBridge C_{18} (5 μm, 4.6 mm × 150 mm) gave sharper peaks and provided the best separation between the azide precursor and [^{18}F]RGD-K5 (resolution 2.18). For the preparative HPLC purification we preferred an isocratic method with a mobile phase consisting of a phosphate buffer in combination with ethanol instead of acetonitrile as was used by Siemens. By using a mobile phase with ethanol as organic modifier we could eliminate the post HPLC SepPak formulation resulting in a reduction of the synthesis time from 90 min (Siemens) to 75 min and a more simple and reliable tracer production [45]. Using the XBridge column in combination with a mobile phase consisting of 12% ethanol in 0.025 M phosphate buffer pH 7, the unreacted azide precursor eluted at 20 min and [^{18}F]RGD-K5 at 25 min. The RGD-K5 azide precursor and the reference compound RGD-K5 have their maximal UV absorption at 210 nm. The reference compound also absorbs at 254 nm, the azide precursor does not. Since for the preparative purification UV detection was performed at 254 nm, the trace of unreacted precursor is not visible in the UV channel of the preparative HPLC chromatogram of the crude radiolabeling mixture (Fig. 2). Within the isocratic conditions unreacted [^{18}F]fluoropentyne is retained on the column and only elutes upon rinsing the HPLC column with EtOH/H_2O 70:30 v/v mixtures. The radiolabeled compound [^{18}F]RGD-K5 was obtained in 35% radiochemical yield based on [^{18}F]fluoride starting radioactivity (decay-corrected) in 75 min.

Analysis of the radiochemical identity, radiochemical and chemical purity and determination of specific radioactivity were performed on

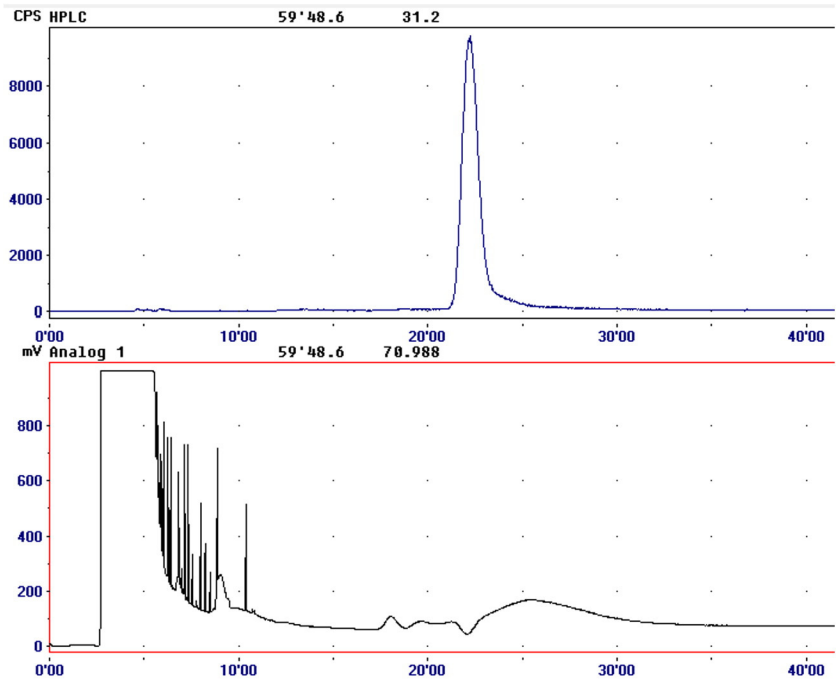


Fig. 2. Semi-preparative HPLC chromatogram of the purification of [^{18}F]RGD-K5. Upper channel: radiometric detection. Lower channel: UV detection at 254 nm. [^{18}F]RGD-K5 elutes at 22 min. Unreacted [^{18}F]fluoropentyne elutes during rinsing of the column.

an analytical HPLC system consisting of a XBridge C_{18} column (3.5 μm, 3 × 100 mm) eluted with 0.025 M Na_2HPO_4 (pH7) and CH_3CN (90:10 v/v). At a flow rate of 0.8 mL/min, [^{18}F]RGD-K5 eluted at 11 min. The cold RGD-K5 standard eluted at the same retention time (11 min ± 1 min) confirming the identity of the tracer. The radiochemical purity was higher than 98% and the specific radioactivity of [^{18}F]RGD-K5 was determined to be in the range of 100–200 GBq/μmol. The absolute amount of cold RGD-K5 was in the range of 15–25 μg. For all productions, QC HPLC analysis (Fig. 3) showed that the amount of RGD-K5 azide precursor (R_t = 6 min) in the final solution was lower than the detection limit (LOD 0.2 ng), confirming the efficient separation between the azide precursor and [^{18}F]RGD-K5 with the applied preparative HPLC system.

The concentration of copper was determined for 5 batches of [^{18}F]RGD-K5 and was found to be 53 ± 22 μg/L which corresponds to 1/10th of the concentration of naturally copper in plasma (50–150 μg/dL) [46]. If the total batch would be injected into a single subject this would result in the administration of <0.5 μg which corresponds to less than 1/1000th of the daily recommended dose (1.2 mg/day), indicating that there is a large safety margin with regard to the copper content in the [^{18}F]RGD-K5 productions. Residual Kryptofix-[2.2.2] analysis was not performed as it was validated that Kryptofix does not co-distil with [^{18}F]fluoropentyne from the first reaction vial. The amount of acetonitrile and o-DCB in the final formulation was below the detection limit (LOD 0.0001%). Ethanol was present (<8%) to increase radiochemical stability and to minimize the tracer being retained on the walls of the sterile filter, the vial and the syringes used to administer the drug product to the patient [45]. The pH of the final formulation was 7.

Radionuclide purity, sterility and endotoxin testing were performed post batch release. The radionuclide identity was determined using a two-time point radioactivity measurement in a dose-calibrator. For all batches, the calculated half-life was in the range 105–115 min which is according to Ph.Eur. guidelines [41]. Bacterial endotoxin determination of the [^{18}F]RGD-K5 batches was done using the Limulus amebocyte lysate (LAL) test according to the Ph.Eur. guidelines [47]. For all batch productions the endotoxin content was <1 IU/mL (limit set at 10 IU/mL). If the total batch volume (max 20 mL) would be injected to one volunteer the amount of injected IU would be well below the 175 IU per dose limit for radiopharmaceuticals specified in the Ph.Eur [47]. Sterility testing was done according to Ph. Eur and no growth of microorganisms was detected after 14 days incubation at 37 °C in any of the batches [48].

By using the CuAAC Click reaction we were able to synthesize [^{18}F]RGD-K5 in good yield under very mild reaction conditions in a reasonable time. By replacing the generally used Cu(I)-stabilizing agents TBTA or diisopropylethyl amine (DiPEA) with Monophos, we could significantly reduce the amount of azide precursor and $CuSO_4$ to ≤0.1 mg. Less precursor results in more efficient preparative HPLC purification with less contamination of the drug product and higher specific radioactivities. Although the decrease of the amount of copper will already reduce the clinical concerns about metal contamination of the radiopharmaceutical, future research should also focus on the application of copper-free click chemistry for the radiosynthesis of biomolecules. The recently developed [^{18}F]AlF method has the advantage that the chelation of the fluoride can be performed in aqueous medium circumventing the time consuming dry-down step and that high specific activities can be obtained after a simple solid phase extraction without chromatographic purification. However the ligand-peptide should be heated up to 100 °C to facilitate the formation of the Al^{18}F-ligand bond, limiting this method to peptides that can tolerate high temperatures. The Monophos Cu(I) click coupling on the other hand can be performed at room temperature, making this method applicable to both

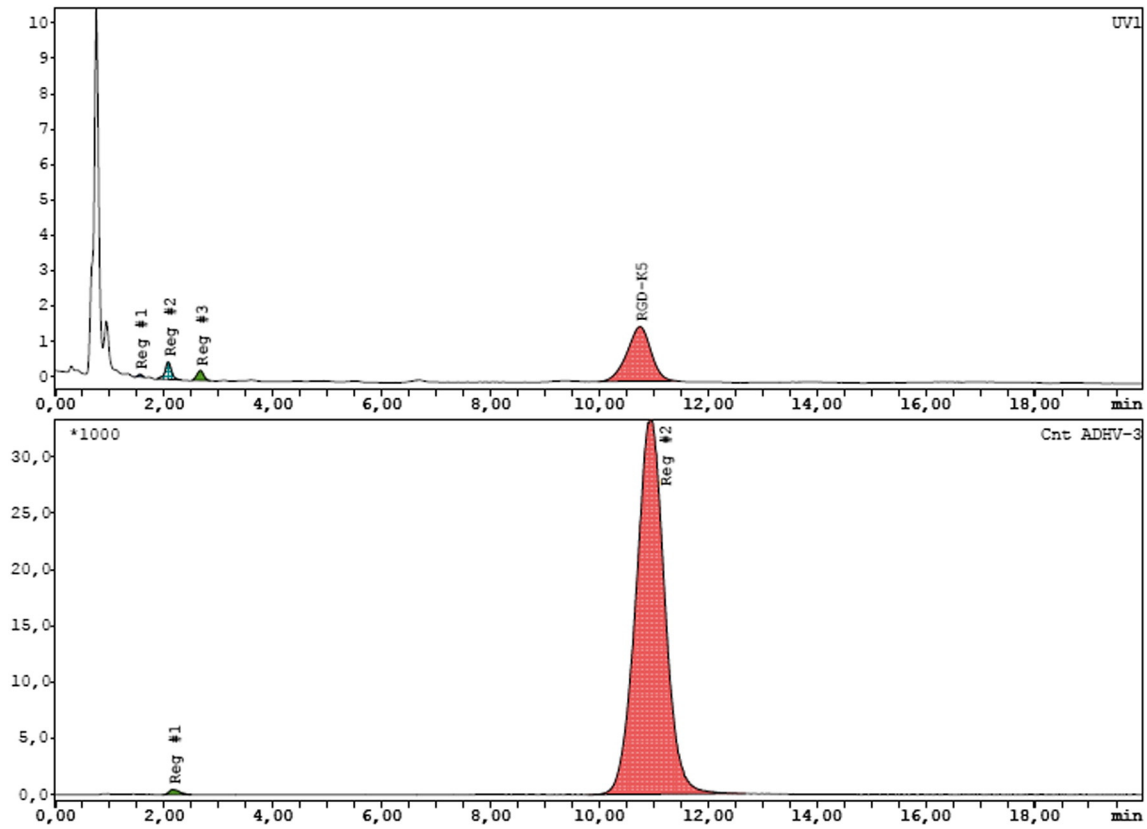


Fig. 3. Quality control of [^{18}F]RGD-K5. Upper channel: UV detection at 210 nm. No trace of RGD-K5 azide precursor is observed at the expected retention time of 6–7 min. Lower channel: radiometric detection.

thermostable and thermolabile proteins and peptides. Derivatization with metal-binding ligands is also applied for the radiolabeling of peptides with gallium-68 [49]. This PET-radioisotope is readily available at a reasonable cost from an in house $^{68}Ge/^{68}Ga$ generator allowing production of these radiolabeled peptides in centers which do not have an on-site cyclotron. Nevertheless, with ^{18}F larger radiotracer batches can be produced allowing more patient studies to be performed per production. A disadvantage of the ^{68}Ga is its high maximum positron energy (1.9 MeV compared to 0.96 MeV for ^{18}F) limiting the spatial resolution of the images and increasing the radiation dose to the patient. Although both the SiFA and the AlF methodology tend more to be true-kit labeling procedures [29,50] compared to the click chemistry approach, the metal-binding ligands that need to be coupled to the peptide are large building blocks, which may influence the molecular integrity, binding properties and immunoreactivity of the peptide or protein. The CuAAC reaction requires less derivatization of the peptide. The triazole linker can been seen as a surrogate for the amide bond and therefore adding a 1,2,3-triazole group is expected not to effect the pharmacological properties of the resulting molecule.

Therefore click chemistry with ^{18}F using the Monophos approach results in a versatile production method for ^{18}F-radiopharmaceuticals. The use of very low amounts of reagents makes the method very suitable if these radiopharmaceuticals are available in small quantities.

4. Conclusion

[^{18}F]RGD-K5 was synthesized with high specific activity and high radiochemical yield using the monophos ligand accelerated Cu(I)-catalyzed 1,3-dipolar cycloaddition reaction. The beneficial effects of this click chemistry for the synthesis of (radiolabeled) biomolecules containing the RGD system will ensure the growth of this area in the future. By applying Monophos as Cu(I) ligand, we were able to optimize and simplify the Siemens method resulting in a shorter synthesis time and a reduction of the amount of reagents. The QC system has been validated and allows the tracer to be used in clinical studies for visualization of neoangiogenesis in oncological patients in our hospitals.

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